

Appl. No. 09/987,025
Amdt. dated February 20, 2004
Reply to Office Action dated October 22, 2003
Atty. Ref. REN-01-020-CON

Amendments to the Claims:

The listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-20 (cancelled).

Claim 21. (previously presented) An isolated nucleic acid molecule comprising a nucleic acid sequence encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase, wherein said 1-deoxy-D-xylulose 5-phosphate reductoisomerase is found in *Arabidopsis*.

Claim 22. (currently amended) An isolated polynucleotide selected from the group consisting of:

(a) an isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2;

(b) an isolated polynucleotide comprising SEQ ID NO: 1;

~~[[c)] an isolated polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 70% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[d)] an isolated polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 80% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[e)] an isolated polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 90% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

[[f)] (c) an isolated polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

[[g)] (d) an isolated polynucleotide that hybridizes[[,]] under stringent conditions to SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; said stringent conditions comprising

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overnight incubation at 42°C in a solution comprising 50% formamide, 5X SSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1X SSC at approximately 65°C; and which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase; and

[[(h)]] (e) an isolated polynucleotide complementary to the polynucleotide sequence of (a), (b), (c), or (d), ~~(e), (f), or (g)~~.

Claim 23. (currently amended) A DNA construct comprising, as operably associated components in the 5' to 3' direction of transcription:

a promoter functional in a plant cell;

a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2;

(b) a polynucleotide comprising SEQ ID NO: 1;

~~[[(c)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 70% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[(d)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 80% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[(e)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 90% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

[[(f)]] (c) a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

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[[[g)]] (d) an isolated polynucleotide that hybridizes[[[,]] under stringent conditions to SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; said stringent conditions comprising overnight incubation at 42°C in a solution comprising 50% formamide, 5X SSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1X SSC at approximately 65°C; and which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase; and

[[[h)]] (e) a polynucleotide complementary to the polynucleotide sequence of (a), (b), (c), or (d), ~~(e), (f), or (g);~~ and,

a transcriptional termination sequence.

Claim 24. (currently amended) A host cell comprising [[a)] the DNA construct of claim 23, comprising, as operably associated components in the 5' to 3' direction of transcription:

— a promoter functional in a plant cell;

— a polynucleotide selected from the group consisting of:

(a) — a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2;

— (b) — a polynucleotide comprising SEQ ID NO: 1;

— (c) — a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 70% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

— (d) — a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 80% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

— (e) — a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 90% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

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~~— (f) — a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~— (g) — a polynucleotide that hybridizes, under stringent conditions to SEQ ID NO: 1 over the entire length of SEQ ID NO: 1 and which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase; and,~~

~~— (h) — a polynucleotide complementary to the polynucleotide sequence of (a), (b), (c), (d), (e), (f), or (g) (d); and,~~

~~— a transcriptional termination sequence.~~

Claim 25. (currently amended) The host cell according to Claim 24, wherein the host cell is a plant cell.

Claim 26. (currently amended) A plant comprising a cell comprising a DNA construct comprising, as operably associated components in the 5' to 3' direction of transcription:

a promoter functional in a plant cell;

a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2;

(b) a polynucleotide comprising SEQ ID NO: 1;

(c) ~~a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 70% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

(d) ~~a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 80% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

(e) ~~a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 90% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

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~~(f)~~ (c) a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

~~[[g]]~~ (d) an isolated polynucleotide that hybridizes~~[[.]]~~ under stringent conditions to SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; said stringent conditions comprising overnight incubation at 42°C in a solution comprising 50% formamide, 5X SSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1X SSC at approximately 65°C; and which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase; and,

~~(h)~~ (e) a polynucleotide complementary to the polynucleotide sequence of (a), (b), (c), ~~(d), (e), (f), or (g)~~ (d); and,

a transcriptional termination sequence.

Claim 27. (currently amended) A method for the alteration of the isoprenoid content in a plant, comprising:

transforming said plant with a construct comprising as operably linked components:

a transcriptional initiation region functional in a plant;

a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2;

(b) a polynucleotide comprising SEQ ID NO: 1;

~~[[c]]~~ a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 70% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

~~[[d]]~~ a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 80% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

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[[[e)]] ~~a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 90% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

[[[f)]] (c) a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

[[[g)]] (d) an isolated polynucleotide that hybridizes[[[,]] under stringent conditions to SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; said stringent conditions comprising overnight incubation at 42°C in a solution comprising 50% formamide, 5X SSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1X SSC at approximately 65°C; and which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase; and

[[[h)]] (e) a polynucleotide complementary to the polynucleotide sequence of (a), (b), (c), or (d), (e), (f), or (g); and,

a transcriptional termination region; and,

growing said plant, wherein said plant has said alteration of isoprenoid content.

Claim 28. (previously presented) The method of Claim 27, wherein said polynucleotide is in the sense orientation.

Claim 29. (previously presented) The method of Claim 28, wherein the isoprenoid content is increased.

Claim 30. (previously presented) The method of Claim 27, wherein said polynucleotide is in the antisense orientation.

Claim 31. (previously presented) The method of Claim 30, wherein the isoprenoid content is decreased.

Claim 32. (currently amended) A method for producing an isoprenoid compound of interest in a plant cell, said method comprising:

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obtaining a transformed plant, said transformed plant having and expressing in its genome:

a primary construct comprising a DNA sequence encoding a polynucleotide comprising a transcriptional initiation region functional in a plant cell operably linked to a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2;

(b) a polynucleotide comprising SEQ ID NO: 1;

~~[[[c)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 70% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[[d)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 80% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[[e)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 90% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

[[[f)] (c) a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

[[[g)] (d) an isolated polynucleotide that hybridizes[[,]] under stringent conditions to SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; said stringent conditions comprising overnight incubation at 42°C in a solution comprising 50% formamide, 5X SSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1X SSC at approximately 65°C; and which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase; and

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[[(h)]] (e) a polynucleotide complementary to the polynucleotide sequence of (a), (b), (c), or (d), ~~(e), (f), or (g)~~; and,

at least one secondary construct comprising a DNA sequence encoding an isoprenoid enzyme operably linked to a transcriptional initiation region functional in a plant cell; and,

growing said plant, wherein said plant produces said isoprenoid compound of interest.

Claim 33. (previously presented) The method of Claim 32, wherein said isoprenoid compound is selected from the group consisting of tocopherols, carotenoids, monoterpenes, diterpenes, and plastoquinones.

Claim 34. (currently amended) A method for increasing the non-mevalonate isoprenoid biosynthetic flux in a cell from a plant, said method comprising:

transforming said plant with a construct comprising as operably linked components:

a transcriptional initiation region functional in a plant;

a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2;

(b) a polynucleotide comprising SEQ ID NO: 1;

~~[[(c)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 70% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[(d)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 80% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

~~[[(e)]] a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 90% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;~~

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[[f]] (c) a polynucleotide comprising a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1;

[[g]] (d) an isolated polynucleotide that hybridizes[[.]] under stringent conditions to SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; said stringent conditions comprising overnight incubation at 42°C in a solution comprising 50% formamide, 5X SSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1X SSC at approximately 65°C; and which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase; and

[[h]] (e) a polynucleotide complementary to the polynucleotide sequence of (a), (b), (c), or (d), ~~(e), (f), or (g)~~; and,

a transcriptional termination region; and,

growing said plant, wherein said plant has an increased non-mevalonate isoprenoid biosynthetic flux.

Claim 35. (cancelled)

Claim 36. (previously presented) An isolated polynucleotide according to Claim 22, wherein said polynucleotide comprises the nucleotide sequence of SEQ ID NO: 1.

Claim 37. (previously presented) An isolated polynucleotide according to Claim 22, wherein said polynucleotide comprises a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2.

Claim 38. (previously presented) An isolated polynucleotide according to Claim 22, wherein said polynucleotide comprises a nucleotide sequence which encodes a 1-deoxy-D-xylulose 5-phosphate reductoisomerase and which has at least 95% identity to that of SEQ ID NO: 1 or its complement, over the entire length of SEQ ID NO: 1.

Claim 39. (previously presented) An isolated DNA comprising a nucleic acid which encodes an *Arabidopsis* 1-deoxy-D-xylulose-5-phosphate reductoisomerase that has the amino acid sequence of SEQ ID NO: 2.